REMARKS

It is generally understood that an uninterrupted stretch of DNA nucleotides is required for an antisense oligonucleotide to elicit the RNaseH activity that is required to meaningfully reduce expression of a target gene *in vivo*. It also understood that beta-D-oxy LNA nucleotides (often simply referred to as LNA) have higher affinity for RNA than do DNA nucleotides. Thus, LNA are often incorporated into antisense oligonucleotides to increase target binding affinity. However, at the time the present application was filed it was also understood that LNA nucleotides do not elicit RNaseH activity, an understanding that arose from the finding the presence of an LNA interrupting a stretch of DNA nucleotides dramatically reduced RNaseH activity. These understandings led to the implementation of the so-called gapmer design for LNA oligonucleotides in which a stretch of DNA nucleotides is flanked by LNA nucleotides. The LNA nucleotides in the "flanks" provide increased target affinity and the DNA nucleotides in the "gap" elicit RNaseH activity.

Despite these common understandings, Applicant surprisingly found that a different type of locked nucleic acid, alpha-L-oxy LNA, can interrupt the stretch of DNA without decreasing the effectiveness of an antisense oligonucleotide. These results, described in the specification (e.g., page 38 and Figure 17), are described in greater detail below.

Amendments to the claims

Claim 45 has been amended to specify that A and C consist of 2 to 5 nucleotide units and that B consists of 5 to 10 nucleotide unites. Support for these amendments is found on pages 8-9 of the published PCT priority application. This claim has also been amended to specify that one or both of A and C comprise at least two consecutive locked nucleotide units. Support for this amendment is found, for example, at page 7, lines 5- 11 and 15 – 21 of the published PCT priority application. New claim 71 is supported in the same manner.

Claim 72 has been added to replace claim 68.

Prior rejections

Applicant appreciates the withdrawal of the enablement rejection under 35 U.S.C. §112, first paragraph.

Election

The recitation of the elections on page 2 of the Office Action is accurate

Obviousness-type double patenting

Claims 43-47, 54-60, 62 and 65-68 were provisionally rejected for obviousness-type double patenting in view of claims 43, 47-51, 53, and 55-96 of co-pending application U.S. Serial No. 10/717,434. Applicant will address this rejection in an appropriate manner upon notification that there are otherwise allowable claims in the present application.

Sequence Listing

The Examiner requested that Applicant submit a sequence listing. However, Applicant does not believed that one is required.

All of the oligonucleotides sequences shown in the above-identified application contain a mixture of 1) nucleotides wherein the sugar component is ribose (RNA); 2) nucleotides wherein the sugar component is 2-deoxyribose (DNA); and 3) nucleotide derivatives wherein the sugar component is a so-called "locked nucleic acid" (i.e., a sugar component which is different from the sugar components found in DNA and RNA).

This application is a US national phase application based on a PCT application. In section 2 of the WIPO Standard ST.25, it is explained that: (ii) Branched sequences, sequences with fewer than four specifically defined nucleotides or amino acids as well as sequences comprising nucleotides or amino acids other than those listed in Appendix 2, Table 1, 2, 3 and 4 are

specifically excluded from this definition; (iii) "nucleotides" embrace only those nucleotides that can be represented using the symbols set forth in Appendix 2, Table 1. Modifications, for example, methylated bases, may be described as set forth in Appendix 2, Table 2, but shall not be shown explicitly in the nucleotide sequence. Thus, under the WIPO Rules no sequence listing is required.

The same is true when one considers the requirements under 37 C.F.R. 1.821 where it states that "(a) Nucleotide and/or amino acid sequences as used in Sec. Sec. 1.821 through 1.825 are interpreted to mean an unbranched sequence of four or more amino acids or an unbranched sequence of ten or more nucleotides. Branched sequences are specifically excluded from this definition. Sequences with fewer than four specifically defined nucleotides or amino acids are specifically excluded from this section. "Specifically defined" means those amino acids other than "Xaa" and those nucleotide bases other than "n" defined in accordance with the World Intellectual Property Organization (WIPO) Handbook on Industrial Property Information and Documentation, Standard ST.25: Standard for the Presentation of Nucleotide and Amino Acid Sequence Listings in Patent Applications (1998), including Tables 1 through 6 in Appendix 2 … herein incorporated by reference. (Hereinafter "WIPO Standard ST.25 (1998)")…Nucleotides: Nucleotides are intended to embrace only those nucleotides that can be represented using the symbols set forth in WIPO Standard ST.25 (1998), Appendix 2, Table 1. Modifications, e.g., methylated bases, may be described as set forth in WIPO Standard ST.25 (1998), Appendix 2, Table 2, but shall not be shown explicitly in the nucleotide sequence." Thus, no sequence listing is required.

Rejections under 35 U.S.C. §103

The Examiner rejected claims 43-47, 54-60, 62 and 65-70 as obvious in view of Kurreck et al. taken with Keinicke et al., Sorensen et al., Orum et al., Wahlestedt et al., and Monia et al.

According to the Examiner, Kurreck teaches LNA/DNA mixmers, gapmers and endblocks, Keinicke teaches that alpha-L-oxy LNA have improved hybridization properties compared to standard DNA oligonucleotides, Sorensen teaches that "α-L-oxy-LNA-containing oligonucleotides are capable of triggering RNase H mediated degradation of a complementary RNA target" and recommend their use as antisense oligonucleotides, and that Orum teaches methods for making and using alpha-L-oxy LNA-containing antisense oligonucleotides. In addition, the Examiner noted that Wahlestedt teaches that an LNA-DNA-LNA gapmer oligonucleotide targeted to a rat opiod receptor was effective in reducing receptor expression. Based on these teachings the Examiner concluded that the present invention would be obvious to one of ordinary skill in the art. Applicant respectfully traverses this rejection.

It is Applicant's position that one skilled in the art would <u>not</u> have arrived at the presently claimed oligonucleotides because one skilled in the art would have expected that an alpha-L-oxy LNA, like a beta-D-oxy LNA, interrupting the stretch of DNA in a gapmer oligonucleotide would sharply diminish the effectiveness of the oligonucleotide as an antisense molecule. Applicant went against this expectation and found that an alpha-L-oxy LNA incorporated in the DNA stretch does not diminish the effectiveness of the oligonucleotide.

One would not include alpha-L-oxy LNA in the gap of a gapmer because LNA decrease RNaseH mediated cleavage when included in the gap

Kurreck conducted an analysis of the effect of gap size on RNaseH-mediated cleavage and found when the contiguous stretch of DNA in a gapmer was less than 8 nucleotides, RNaseHmediated cleavage decreased. Kurreck concluded that "a DNA gap in a chimeric LNA/DNA oligonucleotide is needed to recruit RNase H." (page 1913, right column) Moreover, Kurreck concluded that "a stretch of 7-8 nt in LNA gapmers is needed for full activiation of RNase H". (page 1913, right column) Thus, one skilled in the art would be deterred from including LNA in the gap region of a gapmer because RNAaseH activity, which is very important for the effectiveness of an antisense oligionucleotide, would be diminished. Moreover, to the extent that

one would view alpha-L-oxy LNA to be similar to beta-D-oxy LNA, one would be likewise deterred from including alpha-L-oxy LNA in the gap region of a gapmer.

The view that inclusion of alpha-L-oxy LNA in oligonucleotides is not compatible with efficient RNaseH cleavage is reinforced by Sorensen, who can also be seen as teaching that alpha-L-oxy LNA are not compatible with <u>efficient</u> RNaseH-mediated cleavage. Sorensen reported that an alpha-L-oxy LNA/DNA could elicit RNaseH-mediated cleavage. However, the cleavage was "very slow" and required high enzyme concentrations (page 2164).

It is true that Wahlestedt reports that a LNA/DNA mixmer exhibited some limited ability to recruit RNaseH, but this result shows that the mixmer is much less effective than the DNA/LNA gapmer. This can be seen in Figure 4, which shows a DNA oligonucleotide and a LNA/DNA gapmer cleave the RNA target *in vitro* within 10 minutes. However, the LNA/DNA mixmer elicited almost no cleavage even after 60 minutes. Moreover, even the limited RNaseH cleavage found by Wahlestedt could not be later confirmed. Kurreck, discussed above, stated that they could <u>not</u> confirm the results of Wahlestedt that LNA/DNA mixmers could elicit some RNaseH mediated cleavage (page 1914, first full paragraph).

The cited references, taken as a whole, would deter one from including an alpha-L-oxy LNA in the DNA gap of a gapmer oligonucleotide because doing so would sharply diminish the RNaseH-mediated cleavage that is vital for the *in vivo* effectiveness of an gapmer antisense oligonucleotide.

One would not substitute alpha-L-oxy LNA for beta-D-oxy LNA in the oligonucleotides of the cited references because, according to Keinicke, alpha-L-oxy LNA have reduced affinity for RNA compared to beta-D-oxy LNA

While it is true that certain of the cited references teaching alpha-L-oxy LNA suggest that such nucleotides merit consideration for inclusion in antisense oligonucleotides, none teach that they

should be placed in the gap of gapmer oligonucleotide. Moreover, certain of the references suggest that alpha-L-oxy LNA are not as advantageous as LNA. Thus,Keinicke teaches that incorporation of three alpha-L-oxy LNA into a DNA oligonucleotide increases affinity towards RNA to <u>lesser</u> extent than incorporation of three beta-D-oxy LNA (Table 1 comparing 2 and 3 to 1 and 7 and 8 to 6).

In fact, Keinicke having studied alpha-L-oxy LNA and alpha-L-oxy RNA, suggest that the latter, alpha-L-oxy RNA/alpha-L-oxy LNA chimeras, not alpha-L-oxy LNA/DNA chimeras be studied further for use as antisense molecules (page 595, last paragraph).

Taken as a whole, Keinicke would discourage one from replacing a beta-D-oxy LNA with an alpha-L-oxy LNA because one would expect to see a decrease in binding affinity. This is a second reason that one of ordinary skill in the art would not incorporate an alpha-L-oxy LNA into the gap of a gapmer oligonucleotide.

One skilled in the art would not place an alpha-L-oxy LNA in the gap of a gapmer

The cited prior art, taken as a whole, suggests that one should **not** place an alpha-L-oxy LNA in the gap of a gapmer oligonucleotide because doing should would, like placing an LNA in the gap of a gapmer, reduce RNaseH-mediated cleavage. In addition, the prior art suggests that alpha-L-oxy LNA do not increase affinity towards RNA to the extent that LNA does. For this separate reason, one skilled in the art would not place an alpha-L-oxy LNA in the gap of a gapmer.

Applicant surprisingly found that an alpha-L-oxy LNA placed in the gap of a gapmer does not decrease the effectiveness of the oligonucleotide as an antisense oligonucleotide

Data presented in the present specification demonstrates that, contrary to what would be expected, an alpha-L-oxy-LNA interrupting a run of DNA in an antisense gapmer, does not severely diminish the effectiveness of the antisense gapmer. This can be seen by considering the

antisense gapmers in Table 8 of the present application. The third oligonucleotide listed in Table 8 (4-3-1-3-5a; 2023-t) has 4 beta-D-oxy LNA followed by 3 DNA, 1 alpha-L-oxy LNA, 3 DNA and 5 beta-D-oxy LNA. Thus, this molecule has an <u>uninterrupted stretch of DNA that is only 3 nucleotides long</u>. Based on the behavior of beta-D-oxy-LNA described in the cited references, one would expect that this oligonucleotide, 4-3-1-3-5a , would have poor activity. Despite this expectation, this oligonucleotide significantly reduced expression of a luciferase target by 90% (see Figure 16). In fact, the reduction in luciferase expression was similar to a gapmer oligonucleotide of the same sequence in which the DNA gap was not interrupted by an alpha-L-oxy LNA (i.e., a molecule having a gap containing a run of 7 contiguous DNA; see Figure 16, "gapmer all-PS"). This result is surprising in view of the cited prior art references. An antisense oligonucleotide identical to 4-3-1-3-5a except for the fact that the beta-D-oxy LNA on the inside of each flank were replaced with alpha-L-oxy LNA (4-3-1-3-5b; 2023-u; Table 8, fourth entry) was also very effective (see Figure 16) despite the fact that the uninterrupted stretch of DNA is only 3 nucleotides long. Finally, an oligonucleotide with two alpha-L-oxy LNA replacing DNA in a 9 nucleotide DNA gap, leaving a run of only 5 contiguous DNA, was also effective (see Table 8, second entry (4-1-1-5-1-1-3b; and Figure 16).

The results described in the specification are surprising in view of the prior art, and these results were achieved by doing what the prior suggested should not be done – interrupt the DNA gap region of a gapmer with a locked nucleic acid. Despite the reasonable expectation that such molecules should have sharply reduced efficacy, Applicants found that they are effective.

In view of the forgoing, Applicant respectfully requests that the rejections under 35 U.S.C. §103 be reconsidered and withdrawn.

CONCLUSION

It is believed that the claims are in condition for allowance.

The fees in the amount of $52.00 for excess claim fees and the fees in the amount of $1110.00 for the Petition for Extension of Time are being paid concurrently herewith on the Electronic Filing System (EFS) by way of Deposit Account authorization. Please apply any other charges or credits to Deposit Account No. 06-1050, referencing attorney docket no. 22460-0003US1.

Respectfully submitted,

Date: 22 JUNE 2009

Anita L. Meiklejohn, Ph.D.
Reg. No. 35,283

Fish & Richardson P.C.
225 Franklin Street
Boston, MA 02110
Telephone: (617) 542-5070
Facsimile: (877) 769-7945

22217659.doc